Maximizing the Natriuretic Peptide System in Experimental Heart Failure

Subcutaneous Brain Natriuretic Peptide and Acute Vasopeptidase Inhibition

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Background—A hallmark of congestive heart failure (CHF) is the elevation of the cardiac natriuretic peptides (NPs), which have natriuretic, renin-inhibiting, vasodilating, and lusitropic properties. We have reported that chronic subcutaneous (SQ) administration of brain natriuretic peptide (BNP) in experimental CHF improves cardiorenal function. Vasopeptidase inhibitors (VPIs) are single molecules that simultaneously inhibit both neutral endopeptidase 24.1 (NEP) and ACE. We hypothesized that acute VPI administration would potentiate the cardiorenal actions of SQ BNP in experimental CHF.

Methods and Results—We determined the cardiorenal and humoral responses to acute VPI alone with omapatrilat (OMA) (1 μmol/kg IV bolus) (n=6), acute low-dose SQ BNP (5 μg/kg) alone (n=5), acute VPI plus low-dose SQ BNP (n=5), and acute high-dose SQ BNP (25 μg/kg) alone in 4 groups of anesthetized dogs with experimental CHF produced by ventricular pacing for 10 days. Plasma BNP was greater with VPI+low-dose SQ BNP compared with VPI alone or low-dose SQ BNP alone and was similar to high-dose SQ BNP alone. Urinary BNP excretion was greatest with VPI+SQ BNP. Urinary sodium excretion was also highest with VPI+SQ BNP, with the greatest increase in glomerular filtration rate. VPI+SQ BNP resulted in a greater increase in cardiac output and reduction in cardiac filling pressures as compared with low-dose SQ BNP, high-dose SQ BNP, or VPI alone.

Conclusions—This study reports that acute VPI potentiates the cardiorenal actions of SQ BNP in experimental CHF. This study advances the concept that protein therapy with BNP together with vasopeptide inhibition represents a novel therapeutic strategy in CHF to maximize the beneficial properties of the natriuretic peptide system. (*Circulation*. 2002; 105:999-1003.)

Key Words: natriuretic peptides ■ heart failure ■ pacing

A hallmark of neurohumoral activation in congestive heart failure (CHF) is the plasma elevation of the cardiac natriuretic peptides (NPs) atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP).[1,2] Investigations have demonstrated that these hormones of myocardial cell origin contribute to a maintenance of cardiovascular homeostasis through multiple beneficial actions such as vasodilation, natriuretic, renin-inhibition, and lusitropic properties.[3-5] Indeed, in experimental CHF, pharmacological blockade of the natriuretic peptide receptors results in premature activation of the renin-angiotensin-aldosterone system and sodium retention, therefore demonstrating a compensatory role for the NPs during the development of CHF.[6] Such an important role for these cardiac hormones in cardiorenal regulation is further supported by key studies by Lopez et al,[7] which have demonstrated that genetic deletion of the receptor for ANP and BNP results in an impaired renal response to volume loading and hypertension.

In recent human studies, acute intravenous administration of BNP in subjects with acutely decompensated CHF improved cardiovascular hemodynamics with a reduction in symptoms, underscoring the therapeutic potential of BNP in the treatment of acute heart failure.[8] Complementing these acute studies and with the recognition that BNP is the most potent of the known NPs, we have recently reported that chronic subcutaneous (SQ) administration of BNP in experimental CHF also results in favorable cardiorenal and humoral effects, supporting a strategy for the long-term administration of BNP in CHF.[9]

Vasopeptidase (VP) inhibitors are a new class of cardiovascular agents, which, through a single molecule, simultaneously inhibit the activity of neutral endopeptidase (NEP), which degrades the natriuretic peptides and ACE. The most clinically advanced VP inhibitor is omapatrilat (OMA), which is a mercaptoacyl derivative of a bicyclicthiazepinone

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dipeptide with a molecular weight of 408.5. This single molecule equally inhibits both NEP and ACE, with respective K_i of 8.9 and 6.0 nmol.[10] In experimental CHF, studies have reported that OMA is superior to ACE inhibition in improving mortality rates in the cardiomyopathic hamster.[11] Chen et al[12] have also reported that OMA is superior to ACE inhibition in reducing cardiac filling pressures and enhancing sodium excretion and glomerular filtration rate (GFR) in a canine model of heart failure. Importantly, these actions of VP inhibition (VPI) were attenuated by natriuretic peptide receptor blockade, consistent with the conclusion that the NPs participate in the mechanism of action of OMA. In human CHF, chronic VPI was also superior to ACE inhibition alone in reducing a combined end point of death and hospitalization for worsening CHF.[13] However, in a separate clinical trial, chronic VPI was also associated with a reduction in circulating BNP, presumably due to a reduction in cardiac filling pressures, thus decreasing the stimulus for BNP release by the heart.[14]

On the basis of these previous investigations of the therapeutic efficacy of BNP and VPI in CHF and the knowledge that BNP is a substrate for NEP, we hypothesized that acute VPI would potentiate the cardiorenal actions of SQ BNP, thus representing a therapeutic strategy to maximize the biological actions of the natriuretic peptide system in experimental CHF. To test this hypothesis, we investigated the neurohumoral and cardiorenal responses to acute SQ BNP together with VPI in a canine model of pacing-induced ventricular dysfunction.

Methods

Studies were conducted in four groups of anesthetized male mongrel dogs (weight, 18 to 23 kg; Antech, Inc, Barnhart, Mo) in a characterized model of ventricular dysfunction produced by rapid ventricular pacing at 180 bpm for 10 days on a fixed sodium diet.[15] The four groups consisted of acute VPI alone (n=6), low-dose SQ BNP alone (n=5), VPI plus low-dose SQ BNP (n=5), and high-dose SQ BNP alone (n=5). Studies were performed in accordance with the Animal Welfare Act and with approval of the Mayo Clinic Institutional Animal Care and Use Committee.

Model of Pacing-Induced Heart Failure

All dogs underwent implantation of a programmable cardiac pacemaker (Medtronic). Under pentobarbital sodium anesthesia (30 mg/kg IV) and artificial ventilation (Harvard respirator, Harvard Apparatus) with 5 L/min supplemental oxygen, a left lateral thoracotomy and pericardiotomy were performed. With the heart exposed, a screw-in epicardial pacemaker lead was implanted into the right ventricle. The pacemaker generator was implanted subcutaneously into the left chest wall and connected to the pacemaker lead. Dogs received preoperative and postoperative prophylactic antibiotic treatment with 225 mg clindamycin and 400 000 U procaine penicillin G plus 500 mg dihydrostreptomycin (Combiotic, Pfizer, Inc). Postoperative prophylactic antibiotic was continued through the first 2 postoperative days. Dogs were fed a fixed sodium diet (58 mEq/d, Hill's ID) and allowed water ad lib. All dogs were walked daily. Appetite, activity, body temperature, and condition of surgical skin sites were documented. After a 14-day postoperative recovery period, the pacemaker was turned on at 180 bpm for 10 days. Pacemaker capture was verified by surface ECG every other day.

Acute Protocol

On the day of the acute experiment, dogs were anesthetized with sodium pentobarbital (15 mg/kg IV), intubated, and mechanically ventilated with supplemental oxygen (Harvard respirator) at 16 cycles per minute. A flow-directed, balloon-tipped thermodilution catheter (Ohmeda, Criticath) was advanced to the pulmonary artery through the external jugular vein for cardiac hemodynamic measurement. The femoral artery was cannulated for blood pressure monitoring and blood sampling. The femoral vein was also cannulated for normal saline infusion. The left kidney was exposed by a flank incision, and the ureter was cannulated for urine collection. Supplemental nonhypotensive doses of pentobarbital sodium were given as needed during the experiment.

A 30-minute baseline clearance was performed after a 60-minute equilibration period. Midway through the clearance, cardiac hemodynamics were measured and arterial blood was drawn for hormonal and electrolyte analysis. After the 30-minute baseline clearance, group 1 received an intravenous bolus of the VP inhibitor OMA, 1 μmol/kg administered over 20 minutes. Group 2 received canine BNP, 5 μg/kg administered subcutaneously in the left hind leg only. Group 3 received canine BNP, 5 μg/kg administered subcutaneously in the left hind leg together with intravenous bolus of OMA, 1 μmol/kg administered over 20 minutes. Group 4 received canine BNP, 25 μg/kg administered subcutaneously in the left hind leg only. After drug administration, a 4-hour collection period followed. The doses of 5 μg/kg of BNP and 1 μmol/kg of OMA were chosen, based on our previous studies,[9,12] which showed that we achieved the most favorable cardiorenal effects at these doses. To determine if the more favorable cardiorenal actions of combined VPI plus low-dose SQ BNP could be achieved with higher doses of SQ BNP, we added a high-dose SQ BNP group (25 μg/kg), which resulted in a plasma BNP concentration similar to VPI plus low-dose SQ BNP.

Cardiovascular parameters measured during the acute experiment included mean arterial pressure (MAP), right atrial pressure, pulmonary artery pressure (PAP), cardiac output (CO), and pulmonary capillary wedge pressure (PCWP). CO was determined by thermodilution in triplicate and averaged (Cardiac Output model 9510-A computer, American Edwards Laboratories). MAP was assessed by direct measurement from the femoral arterial catheter.

Urine was collected on ice for assessment of urine volume, electrolytes, and inulin. Urine collected for cGMP analysis was heated to >90°C before storage. Arterial blood was collected in heparin and EDTA tubes and immediately placed on ice. After centrifugation at 2500 rpm at 4°C, plasma was decanted and stored at −20°C until analysis.

Hormone and Electrolyte Analysis

After plasma extraction, plasma and urine ANP and BNP were measured by radioimmunoassay (RIA), as previously described.[16,17] Plasma and urinary samples for cGMP were measured by RIA with the method of Steiner et al.[18] Plasma renin activities and aldosterone were determined by RIA with the methods of Haber et al.[19,20] Urinary and plasma inulin concentrations were measured by the anthrone method.

Statistical Analysis

Results of the quantitative studies are expressed as mean±SEM. Data were assessed by repeated-measures 1-way ANOVA for comparisons within groups; 2-way ANOVA was used for comparison between groups, with the use of GraphPad Prism software. Statistical significance was accepted as $P<0.05$.

Results

Humoral, Renal, and Cardiovascular Actions of VPI Alone Versus Low-Dose SQ BNP Alone Versus VPI+Low-Dose SQ BNP

Figure 1 illustrates the responses of plasma and urinary BNP excretion and urinary sodium excretion in the VPI alone, low-dose SQ BNP, and VPI+low-dose SQ BNP groups. Both VPI alone and low-dose SQ BNP alone increased plasma BNP; however, VPI+SQ BNP resulted in the greatest in-
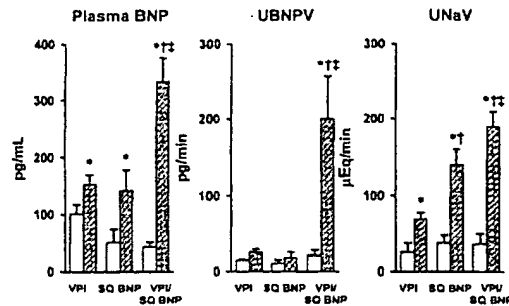
Figure 1. Plasma BNP, urinary BNP excretion (UBNPV), and urinary sodium excretion (UNaV) at baseline (open bars) and mean values (hatched bars) after VPI alone, low-dose SQ BNP alone, and VPI+low-dose SQ BNP administration. *P<0.05 vs baseline, †P<0.05 vs VPI alone, ‡P<0.05 vs low-dose SQ BNP alone.

crease in plasma BNP as compared with either treatment. The greater increase in plasma BNP with VPI+SQ BNP was associated with an increase in urinary BNP excretion, which was observed neither with SQ BNP alone nor with VPI alone. Both plasma cGMP (VPI alone, 21±4; low-dose SQ BNP alone, 15.9±2; VPI+low-dose SQ BNP, 16.3±2 pmol/mL; $P>0.05$) and urinary cGMP excretion (VPI alone, 2233±326; low-dose SQ BNP alone, 2161±232; VPI+low-dose SQ BNP, 2595±230 pmol/min; $P>0.05$) were similar in all three groups. Urinary sodium excretion increased in response to VPI alone (Figure 1). Low-dose SQ BNP alone increased sodium to a higher level as compared with VPI alone. Finally, combined VPI+SQ BNP increased urinary sodium excretion to a level greater than that observed in the two other groups. The increase in GFR was the greatest in VPI+SQ BNP as compared with VPI alone or SQ BNP alone (28±5 versus 13±3 versus 8±5 mL/min, respectively; $P<0.05$, 2-way ANOVA), whereas renal blood flow (VPI alone, 214±29; low-dose SQ BNP alone, 225±30; VPI+low-dose SQ BNP, 238±34 mL/min; $P>0.05$) increased to a similar extent in all three groups.

Figure 2 illustrates the cardiovascular hemodynamic responses in VPI alone, low-dose SQ BNP, and VPI+low-dose SQ BNP groups. VPI alone produced a significant increase in cardiac output that was not observed with low-dose SQ BNP
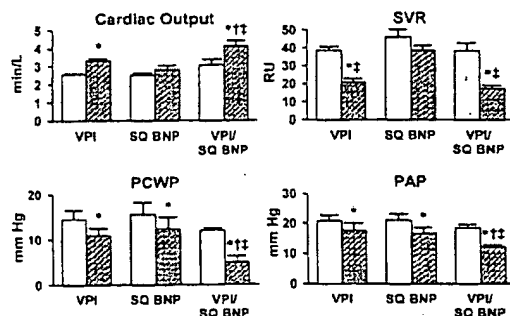

Figure 2. Cardiac output, SVR, PCWP, and PAP at baseline (open bars) and peak effects (hatched bars) after VPI alone, low-dose SQ BNP alone, and VPI+low-dose SQ BNP administration. *P<0.05 vs baseline, †P<0.05 vs VPI alone, ‡P<0.05 vs low-dose SQ BNP alone.

alone. However, VPI+low-dose SQ BNP increased cardiac output to a level greater than the response observed with either VPI or low-dose SQ BNP alone. In response to VPI alone, systemic vascular resistance (SVR) decreased significantly to a level greater than that which occurred with low-dose SQ BNP alone, and this response was similar to that with combined VPI+low-dose SQ BNP. Both PCWP and PAP decreased similarly to VPI alone and low-dose SQ BNP alone. The level of reduction in PCWP and PAP with VPI+low-dose SQ BNP was greater than that achieved with either VPI alone or low-dose SQ BNP alone. Mean arterial blood pressure was reduced to a similar magnitude in both the VPI alone and VPI+low-dose SQ BNP groups (103±4 to 72±7 mm Hg with VPI alone, $P<0.05$; 116±4 to 76±5 mm Hg, VPI+SQ BNP, $P<0.05$), whereas it remained unchanged with SQ BNP alone (114±9 to 110±9 mm Hg).

Humoral, Renal, and Cardiovascular Actions of VPI+Low-Dose SQ BNP Versus High-Dose SQ BNP Alone

To determine if the more favorable cardiorenal actions of combined VPI plus low-dose SQ BNP could be achieved with a higher dose of SQ BNP, we added a high-dose SQ BNP group (25 μg/kg), which resulted in a plasma BNP concentration similar to VPI+low-dose SQ BNP. The Table reports the comparison between VPI+low-doses SQ BNP versus high-dose SQ BNP alone. Despite achieving similar plasma BNP concentrations, urinary BNP excretion was greater in the VPI+low-dose SQ BNP group as compared with the high-dose SQ BNP alone group. Urinary sodium excretion and CO were also greater in the VPI+low-dose SQ BNP group as compared with the high-dose SQ BNP alone group. There was a greater reduction in MAP, SVR, and PAP in the VPI+low-dose SQ BNP group as compared with the high-dose SQ BNP alone group. PCWP tended to be lower in the VPI+low-dose SQ BNP group as compared with the high-dose SQ BNP alone group, but this did not reach statistical significance.

Discussion

The present study sought to define the cardiorenal and neurohumoral actions of dual therapy with low-dose SQ BNP and acute VPI in experimental CHF, testing the hypothesis that simultaneous inhibition of NEP and ACE with VPI would potentiate the beneficial cardiorenal actions of the cardiac hormone BNP. This study demonstrates that acute VPI with OMA, together with subcutaneous administration of the cardiac hormone BNP in experimental CHF, resulted in a greater increase in plasma BNP and urinary BNP excretion as compared with both VPI alone or low-dose SQ BNP alone. These actions were associated with a greater natriuretic response, greater increases in CO and GFR, and greater decrease in cardiac filling pressures. Furthermore, these favorable cardiorenal and humoral actions of combined VPI and low-dose SQ BNP were not achieved with a higher dose of SQ BNP, which reached similar plasma BNP levels, therefore suggesting a synergistic action between VPI and BNP. This study advances the concept that low-dose SQ BNP, together with VP inhibition, represents a therapeutic

Comparison Between VPI and Low-Dose SQ BNP Versus High-Dose SQ BNP Alone

	VPI and Low-Dose SQ BNP	High-Dose SQ BNP Alone
Plasma BNP, pg/mL		
Baseline	45±13	41±6
After	307±40*	308±56*
UBNPV, pg/min		
Baseline	21±13	14±3
After	200±56*†	40±7*
UNaV, μEq/min		
Baseline	35±14	18±8
After	181±19*†	117±20*
CO, L/min		
Baseline	2.9±0.3	2.5±0.2
After	4.1±0.3*†	3.0±0.3*
MAP, mm Hg		
Baseline	116±4	115±4
After	76±5*†	96±4*
SVR, RU		
Baseline	38±4	43±3
After	17±1*†	31±3*
PCWP, mm Hg		
Baseline	12±1	13±1
After	5±1*	8±1*
PAP, mm Hg		
Baseline	19±0.7	19±0.8
After	13±0.2*†	16±0.8*

Values are mean±SEM. UBNPV indicates urinary BNP excretion; and UNaV, urinary sodium excretion.

*$P<0.05$ vs baseline; †$P<0.05$ vs high-dose SQ BNP alone.

strategy in CHF that maximizes the beneficial properties of the natriuretic peptide system.

Both VPI alone and low-dose SQ BNP alone increased plasma BNP to a similar extent, the former by inhibiting its degradation and the latter by adding exogenous peptide. In addition, VPI+low-dose SQ BNP and high-dose SQ BNP resulted in similar increases in plasma BNP, which was significantly higher as compared with VPI alone or low-dose SQ BNP alone. Despite the increase in plasma BNP with VPI alone and low-dose SQ BNP alone, there was no increase in urinary BNP excretion. With high-dose SQ BNP there was an increase in urinary BNP excretion; however, this was significantly less than that achieved with VPI+low-dose SQ BNP. This reduced level of increase in urinary BNP excretion with high-dose SQ BNP despite similar plasma levels as observed in the VPI+low-dose SQ BNP is most likely due to the degradation of BNP by NEP, which is abundant in the kidney. Therefore, the much greater increase in urinary BNP excretion in the VPI+low-dose SQ BNP group probably is due to the synergistic effects of increasing plasma BNP and inhibition of renal NEP and ACE by VPI. The functional importance of this greater increase in plasma and urinary BNP excretion is the greater increase in natriuresis and GFR

despite a greater decrease in MAP seen with VPI+low-dose SQ BNP.

With low-dose SQ BNP, there was no change in MAP, CO, and SVR, whereas with VPI alone CO increased with decreases in MAP and SVR. Importantly, with VPI+low-dose SQ BNP, CO increased further compared with VPI alone, whereas SVR decreased similar to VPI alone. Furthermore, VPI+low-dose SQ BNP resulted in the greatest reduction in PCWP and PAP as compared with low-dose SQ BNP alone or VPI alone. These changes in hemodynamic effects probably are due to both NEP and ACE inhibition and the increase in plasma BNP level observed with VPI+low-dose SQ BNP. Indeed, as we have demonstrated, the administration of the NP receptor antagonist HS 142-1 attenuated the favorable cardiorenal effects of VPI in experimental CHF.[12] Most intriguing is the speculation that the greater increase in CO with VPI+low-dose SQ BNP despite greater reductions in cardiac filling pressures and similar reductions in SVR suggest a positive inotropic response. Indeed, in conscious dogs, both BNP and ANP have been reported to have inotropic as well as lusitropic actions.[21] Further evidence to suggest that the favorable cardiovascular hemodynamics seen with VPI+low-dose SQ BNP is due to the synergistic actions of both NEP inhibition and ACE inhibition is the fact that despite similar plasma BNP levels, CO was greater and MAP, SVR, and PAP were lower in the VPI+low-dose SQ BNP group as compared with the high-dose SQ BNP alone group.

The present study may have important therapeutic implications. Attempts have recently been made to promote activation or restoration of endogenous vasodilating natriuretic and myocardial modulating systems in the management of CHF. Indeed, a major goal has been to develop agents to unload the heart, resulting in reverse remodeling. The FDA has recently approved intravenous human BNP as therapy for acute decompensated heart failure. Studies have reported greater blood pressure–reducing properties of OMA in human hypertension when compared with conventional antihypertensive agents such as ACE inhibitors.[22] Furthermore, in a small clinical trial in patients with symptomatic CHF (IMPRESS), OMA was reported to be superior to ACE inhibition in reducing a combined end point of worsening heart failure and hospitalization compared with ACE inhibition alone. In IMPRESS, an important property of OMA was improving renal function as compared with ACEI[13]; however, the beneficial effects of OMA may not be entirely realized in association with chronic reduction of endogenous NP production secondary to decreases in cardiac filling pressures.[14]

We have reported in previous studies the beneficial effects of repeated short-term SQ BNP administration in experimental CHF and that acute SQ BNP has favorable cardiorenal and neurohumoral actions in human CHF. Therefore, the present study, although performed in a small number of animals, supports the conclusion that VP inhibition and SQ BNP administration has both additive and synergistic cardiorenal actions in experimental heart failure. Further investigations with chronic SQ BNP+VP inhibition as a therapeutic strategy for the management of heart failure are clearly warranted.

In conclusion, this study reports for the first time that VPI potentiates the cardiorenal actions of SQ BNP in experimen-

tal CHF. This study advances the novel concept that cardiac hormone protein therapy with SQ BNP together with VP inhibition represents a therapeutic strategy in CHF to maximize the beneficial properties of the natriuretic peptide system by simultaneously inhibiting ACE and NEP and providing additional BNP, which is a key substrate for NEP.

Acknowledgments

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